| | Type | L # | Hits | Search Text | DBs | Time Stamp | Comments | Error Definition | Errors |
|---|---|---|---|---|---|---|---|---|---|
| 1 | BRS | L1 | 4167 | molecul$ same switch | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 11:54 | | | 0 |
| 2 | BRS | L2 | 459 | molecular adj switch | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:15 | | | 0 |
| 3 | BRS | L3 | 13469 | (transcription adj factor) or (transcriptional adj regulatory adj protein) or (transcriptional adj regulatory adj factor) or (DNA adj binding adj protein) | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:16 | | | 0 |
| 4 | BRS | L4 | 9740 | vp16 or NF-kappaB or Gal4 or tfe3 or itf1 or oct-1 or sp1 or oct-2 or nfy-A or itf2 or c-cmc or ctf | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:21 | | | 0 |
| 5 | BRS | L5 | 295759 | kruppel or krab or kox-1 or tetr or even-skipped or lacr or engrailed or ebna3 or mad or v-erba or hairy or hes or groucho or tle or ring1 or ssb16 or ssb24 or tup1 or nab1 or areb or e4bp4 or hoxa7 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:29 | | | 0 |
| 6 | BRS | L6 | 312858 | 3 or 4 or 5 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:30 | | | 0 |
| 7 | BRS | L7 | 311 | DNA adj binding adj (element or sequence) | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:31 | | | 0 |
| 8 | BRS | L8 | 101775 | promoter | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:31 | | | 0 |

| | Type | L # | Hits | Search Text | DBs | Time Stamp | Comments | Error Definition | Errors |
|---|---|---|---|---|---|---|---|---|---|
| 9 | BRS | L9 | 2464376 | molecule or compound or ligand or inducer | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:32 | | | 0 |
| 10 | BRS | L10 | 40028 | gene adj express$3 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:32 | | | 0 |
| 11 | BRS | L11 | 10264 | transgene | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:33 | | | 0 |
| 12 | BRS | L12 | 0 | 2 same 6 same 7 same 8 same 9 same 11 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:34 | | | 0 |
| 13 | BRS | L13 | 0 | 2 same 6 same 7 same 8 same 9 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:35 | | | 0 |
| 14 | BRS | L14 | 0 | 2 same 6 same 7 same 8 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:35 | | | 0 |
| 15 | BRS | L15 | 0 | 2 same 11 same 7 same 8 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:36 | | | 0 |
| 16 | BRS | L16 | 0 | 10 same 6 same 7 same 8 same 11 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:37 | | | 0 |
| 17 | BRS | L17 | 3 | 10 same 6 same 7 same 8 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:42 | | | 0 |
| 18 | BRS | L18 | 0 | non-native adj binding adj (sequence or site) | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:43 | | | 0 |
| 19 | BRS | L19 | 16 | non-native adj DNA adj (sequence or site) | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:43 | | | 0 |
| 20 | BRS | L20 | 0 | (2 or 10) same 19 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:44 | | | 0 |
| 21 | BRS | L21 | 0 | 1 same 19 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:45 | | | 0 |
| 22 | BRS | L22 | 0 | lim adj moon adj young.in. | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:46 | | | 0 |
| 23 | BRS | L23 | 13 | edwards adj cynthia.in. | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:46 | | | 0 |
| 24 | BRS | L24 | 33 | fry adj kirk.in. | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:47 | | | 0 |

| | Type | L # | Hits | Search Text | DBs | Time Stamp | Comments | Error Definition | Errors |
|---|---|---|---|---|---|---|---|---|---|
| 25 | BRS | L25 | 34 | bruice adj thomas.in. | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:47 | | | 0 |
| 26 | BRS | L26 | 1 | starr adj douglas.in. | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:48 | | | 0 |
| 27 | BRS | L27 | 4 | laurance adj megan.in. | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:48 | | | 0 |
| 28 | BRS | L28 | 12 | kwok adj yan.in. | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:48 | | | 0 |
| 29 | BRS | L29 | 84 | 23 or 24 or 25 or 26 or 27 or 28 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:49 | | | 0 |
| 30 | BRS | L30 | 0 | 29 and 2 | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/03/10 12:49 | | | 0 |

=> d his

(FILE 'HOME' ENTERED AT 12:54:51 ON 10 MAR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
12:55:38 ON 10 MAR 2003
L1 3988 S MOLECULAR SWITCH
L2 35784 S MODULAT? (P) (GENE EXPRESSION)
L3 391144 S (TRANSCRIPTION? FACTOR) OR (TRANSCRIPTION? REGULATORY PROTEIN
L4 46070 S VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 ORO
L5 74633 S VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 OR
L6 62751 S KRUPPEL OR KRAB OR KOX-1 OR TETR OR EVEN-SKIPPED OR LACR OR E
L7 1568 S RING1 OR SSB16 OR SSB24 OR TUP1 OR NAB1 OR AREB OR E4BP4 OR H
L8 137527 S L5 OR L6 OR L7
L9 71820 S TRANSGENE
L10 26 S (L1 OR L2) (P) L8 (P) L9
L11 243160 S (DNA BINDING)
L12 543375 S PROMOTER
L13 775 S (L1 OR L2) (P) L11 (P) L12
L14 5552 S NON-NATIVE
L15 0 S L13 (P) L14
L16 0 S L10 (P) L14
L17 10 DUPLICATE REMOVE L10 (16 DUPLICATES REMOVED)
L18 4 S L17 (P) L12 (P) L11
L19 6 S L17 NOT L18

=> log y

```
FILE 'MEDLINE' ENTERED AT 12:55:38 ON 10 MAR 2003

FILE 'CAPLUS' ENTERED AT 12:55:38 ON 10 MAR 2003
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2003 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 12:55:38 ON 10 MAR 2003
COPYRIGHT (C) 2003 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'EMBASE' ENTERED AT 12:55:38 ON 10 MAR 2003
COPYRIGHT (C) 2003 Elsevier Science B.V.  All rights reserved.

FILE 'SCISEARCH' ENTERED AT 12:55:38 ON 10 MAR 2003
COPYRIGHT (C) 2003 Institute for Scientific Information (ISI) (R)

FILE 'AGRICOLA' ENTERED AT 12:55:38 ON 10 MAR 2003

=> s molecular switch
L1         3988 MOLECULAR SWITCH

=> s modulat3 (p) (gene expression)
3 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=>  s modulat? (p) (gene expression)
   3 FILES SEARCHED...
L2        35784 MODULAT? (P) (GENE EXPRESSION)

=> s (transcription? factor) or (transcription? regulatory protein) or (transcription? regulatory
   3 FILES SEARCHED...
   5 FILES SEARCHED...
L3       391144 (TRANSCRIPTION? FACTOR) OR (TRANSCRIPTION? REGULATORY PROTEIN)
                OR (TRANSCRIPTION? REGULATORY FACTOR) OR (DNA BINDING PROTEIN)

=> s vp16 or NF-kappaB or gal4 or tfe3 or itf1 or oct-1 or sp1 oroct-2 or nfy-a or itf2 orc-cmc or
L4        46070 VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 OROCT-
                2 OR NFY-A OR ITF2 ORC-CMC OR CTF

=> s vp16 or NF-kappaB or gal4 or tfe3 or itf1 or oct-1 or sp1 or oct-2 or nfy-a or itf2 or c-cmc
L5        74633 VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 OR
                OCT-2 OR NFY-A OR ITF2 OR C-CMC OR CTF

=> s kruppel or krab or kox-1 or tetr or even-skipped or lacr or engrailed or ebna3 or mad or v-er
L6        62751 KRUPPEL OR KRAB OR KOX-1 OR TETR OR EVEN-SKIPPED OR LACR OR
                ENGRAILED OR EBNA3 OR MAD OR V-ERBA OR HAIRY OR HES OR GROUCHO
                OR TLE

=> s ring1 or ssb16 or ssb24 or tup1 or nab1 or areb or e4bp4 or hoxa7
L7         1568 RING1 OR SSB16 OR SSB24 OR TUP1 OR NAB1 OR AREB OR E4BP4 OR
                HOXA7

=> s l5 or l6 or l7
L8       137527 L5 OR L6 OR L7

=> d his

     (FILE 'HOME' ENTERED AT 12:54:51 ON 10 MAR 2003)

     FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
     12:55:38 ON 10 MAR 2003
L1         3988 S MOLECULAR SWITCH
L2        35784 S MODULAT? (P) (GENE EXPRESSION)
L3       391144 S (TRANSCRIPTION? FACTOR) OR (TRANSCRIPTION? REGULATORY PROTEIN
L4        46070 S VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 ORO
L5        74633 S VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 OR
L6        62751 S KRUPPEL OR KRAB OR KOX-1 OR TETR OR EVEN-SKIPPED OR LACR OR E
L7         1568 S RING1 OR SSB16 OR SSB24 OR TUP1 OR NAB1 OR AREB OR E4BP4 OR H
```

```
L8       137527 S L5 OR L6 OR L7

=> s transgene
L9        71820 TRANSGENE

=> s (l1 or l2) (p) l8 (p) l9
L10          26 (L1 OR L2) (P) L8 (P) L9

=> s (DNA binding)
L11      243160 (DNA BINDING)

=> s promoter
L12      543375 PROMOTER

=> s (l1 or l2) (p) l11 (p) l12
L13         775 (L1 OR L2) (P) L11 (P) L12

=> s non-native
L14        5552 NON-NATIVE

=> s l13 (p) l14
L15           0 L13 (P) L14

=> s l10 (p) l14
L16           0 L10 (P) L14

=> duplicate remove l10
DUPLICATE PREFERENCE IS 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L10
L17           10 DUPLICATE REMOVE L10 (16 DUPLICATES REMOVED)

=> s l17 (p) l12 (p) l11
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L118 (P) L80'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L80 (P) L73'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L120 (P) L81'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L81 (P) L74'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L124 (P) L83'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L83 (P) L76'
L18           4 L17 (P) L12 (P) L11

=> d l18 1-4 ibib abs

L18  ANSWER 1 OF 4     MEDLINE
ACCESSION NUMBER:   2002635673     MEDLINE
DOCUMENT NUMBER:    22282001   PubMed ID: 12395191
TITLE:              A Renilla luciferase-Aequorea GFP (ruc-gfp) fusion gene
                    construct permits real-time detection of promoter
                    activation by exogenously administered mifepristone in
                    vivo.
AUTHOR:             Yu Y A; Szalay A A
CORPORATE SOURCE:   Division of Human Anatomy, Loma Linda University School of
                    Medicine, Loma Linda, CA 92350, USA.
SOURCE:             Mol Genet Genomics, (2002 Oct) 268 (2) 169-78.
                    Journal code: 101093320. ISSN: 1617-4615.
PUB. COUNTRY:       Germany: Germany, Federal Republic of
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200212
ENTRY DATE:         Entered STN: 20021024
                    Last Updated on STN: 20030105
                    Entered Medline: 20021213
AB   In this study, we used a steroid-induced   ***promoter***   activation
     system as a   ***molecular***     ***switch***   to study the exogenous
     activation of   ***transgene***   expression. This   ***promoter***
```

activation system consists of three components: (1) a steroidal inducer drug, mifepristone (RU486), which binds to (2) a chimeric transcription factor complex, consisting of the mutant human progesterone receptor fused to the yeast ***GAL4*** ***DNA*** - ***binding*** domain and the activation domain of the herpes simplex virus protein ***VP16*** , and (3) a synthetic ***promoter*** , consisting of a series of ***GAL4*** recognition sequences upstream of the adenovirus major late E1B TATA box, linked to a gene construct (ruc-gfp) encoding a Renilla luciferase-Aequorea green fluorescent protein (GFP) fusion protein. Transcription of the ***promoter*** -marker gene cassette is activated by the drug (mifepristone)-bound chimeric transcription factor complex. Monitoring of induced gene expression was carried out using a low-light video camera and a UV microscope to detect luciferase and GFP, respectively. Using this activation system, we observed a 10- to 25-fold activation, depending on the inducer dose, of both luciferase and GFP expression in transiently transfected cells in comparison to cells that were not exposed to mifepristone. We further demonstrated activation of gene expression from the ***promoter*** activation system in live animals. The plasmids PAP CMV-GL914VPc'SV, carrying the chimeric transcription factor cassette, and plasmid p17x4-TATA-ruc-gfp, carrying the ruc-gfp reporter gene construct, were co-injected into limb muscles of nude mice. Following DNA injection, mifepristone (50 micro g/kg) was delivered by intraperitoneal injection. Thirty-six hours after DNA and mifepristone injection, significant Renilla luciferase activity was detectable in the limb muscles. The ***promoter*** activation system was also demonstrated in limb muscles and livers of nude mice that had received transplants of ex vivo-modified cells, which were transiently transformed with both the chimeric activator plasmid and the ruc-gfp reporter plasmid prior to implantation. Significant Renilla activity and GFP fluorescence were detected externally in limb muscles and in the livers of anesthetized animals that had received an intraperitoneal injection of inducer. This external monitoring method for observing inducible gene expression in live animals will facilitate experimental studies of fundamental questions of biological and therapeutic relevance. It will be especially valuable for the analysis of gene function at specific stages of animal development. The method should also be of general use in gene therapy, since it permits simultaneous monitoring of the expression levels of light-emitting proteins and therapeutic proteins originating from the activation of identical ***promoters*** .

L18 ANSWER 2 OF 4 MEDLINE
ACCESSION NUMBER: 1999348185 MEDLINE
DOCUMENT NUMBER: 99348185 PubMed ID: 10417731
TITLE: Ecdysone agonist inducible transcription in transgenic tobacco plants.
AUTHOR: Martinez A; Sparks C; Hart C A; Thompson J; Jepson I
CORPORATE SOURCE: ZENECA Agrochemicals, Jealott's Hill Research Stsation, Bracknell, Berkshire, UK.. Alberto.Martinez@AGUK.Zeneca.com
SOURCE: PLANT JOURNAL, (1999 Jul) 19 (1) 97-106.
Journal code: 9207397. ISSN: 0960-7412.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Y09009
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991014
Last Updated on STN: 19991014
Entered Medline: 19991005

AB A novel chemical-induced gene regulatory system for plants consisting of two molecular components is described. The first, or regulatory, cassette comprises a chimeric receptor composed of the hinge and ligand binding domains of the Heliothis virescens ecdysone receptor and the transactivation domain of the Herpes simplex ***VP16*** protein fused to the ***DNA*** ***binding*** domain and transactivation of a mammalian glucocorticoid receptor. The second component, a reporter cassette, contains six copies of the glucocorticoid response element (GRE) fused to the minimal 35SCaMV ***promoter*** and beta-glucuronidase. The system uses a commercially available non-steroidal ecdysone agonist, RH5992 (tebufenozide), as an inducer. Activation of ***gene*** ***expression*** is shown in both tobacco transient protoplasts and transgenic plants. The response is ligand dependent and is

***modulated*** by the change in minimal ***promoter*** context. The system is capable of inducing ***transgene*** activity up to 420-fold corresponding to 150% of the activity observed with positive controls (35SCaMV:GUS).

L18 ANSWER 3 OF 4 MEDLINE
ACCESSION NUMBER: 97172502 MEDLINE
DOCUMENT NUMBER: 97172502 PubMed ID: 9020146
TITLE: Nuclear factor interleukin 6 motifs mediate tissue-specific gene transcription in hypoxia.
AUTHOR: Yan S F; Zou Y S; Mendelsohn M; Gao Y; Naka Y; Du Yan S; Pinsky D; Stern D
CORPORATE SOURCE: Departments of Physiology, Surgery, Medicine, and Pathology, Columbia University, College of Physicians and Surgeons, New York, New York 10032, USA.
CONTRACT NUMBER: HL42507 (NHLBI)
HL50629 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Feb 14) 272 (7) 4287-94.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970327
Last Updated on STN: 19970327
Entered Medline: 19970314
AB Activation of transcription at the nuclear factor interleukin 6 (NF-IL-6) ***DNA*** ***binding*** motif ***modulates*** expression of multiple genes important in host adaptive and developmental mechanisms. Studies showing that hypoxia-induced transcription of IL-6 in cultured endothelial cells was due to transcriptional activation by the NF-IL-6 motif in the ***promoter*** (Yan, S.-F., Tritto, I., Pinsky, D., Liao, H., Huang, J., Fuller, G., Brett, J., May, L., and Stern, D. (1995) J. Biol. Chem. 270, 11463-11471) led us to prepare transgenic mice using 115- or 14-base pair regions of the ***promoter*** encompassing the NF-IL-6 site ligated to the lacZ reporter gene and the basal thymidine kinase ***promoter*** . On exposure to hypoxia or induction of ischemia, mice bearing either of the constructs showed prominent expression of the ***transgene*** in lung and cardiac vasculature and in the kidney but not in the liver (parenchyma or vasculature). In contrast, transgenic mice bearing a mutationally inactivated NF-IL-6 site showed no increase in ***transgene*** expression in hypoxia. Gel retardation assays revealed time-dependent, hypoxia-enhanced nuclear binding activity for the NF-IL-6 site in nuclear extracts of the heart, lung, and kidney but not in the liver; the hypoxia-enhanced band disappeared on addition of antibody to C/EBPbeta-NF-IL-6. Consistent with the specificity of hypoxia-mediated activation of C/EBPbeta-NF-IL-6, gel retardation assays showed no change in the intensity of the hypoxia-enhanced gel shift band in the presence of excess unlabeled oligonucleotide probes or antibodies related to other transcription factors, including NFkappaB, AP1, cAMP response element-binding protein, ***SP1*** , and hypoxia-inducible factor 1. These data indicate that the transcription factor NF-IL-6 is sensitive to environmental oxygen deprivation, and the tissue-specific pattern of ***gene*** ***expression*** suggests that local mechanisms have an important regulatory effect.

L18 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:240960 CAPLUS
DOCUMENT NUMBER: 136:274272
TITLE: Ligand-dependent regulation of transgene expression by a plasmid-based autoinducible GeneSwitch system for gene therapy application
INVENTOR(S): Abruzzese, Ronald V.; Mehta, Vidya; Nordstrom, Jeffrey L.
PATENT ASSIGNEE(S): Valentis, Inc., USA
SOURCE: PCT Int. Appl., 101 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2002024899 | A2 | 20020328 | WO 2001-US30305 | 20010925 |
| WO 2002024899 | A3 | 20021212 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

| | | | | |
|---|---|---|---|---|
| AU 2001096354 | A5 | 20020402 | AU 2001-96354 | 20010925 |

PRIORITY APPLN. INFO.:

US 2000-235030P P 20000925
US 2001-260781P P 20010110
US 2001-278281P P 20010323
WO 2001-US30305 W 20010925

AB The present invention provides an improved ***mol*** .- ***switch*** , inducible-expression system for regulating the expression of a nucleic acid sequence in gene therapy under conditions in which tight control of expression is of particular importance. In one aspect of the invention, a system is provided wherein expression of the gene to be induced is characterized by low or undetectable expression or biol. effect in the absence of the inducer, but in the presence of the inducer, is characterized by efficient induction of expression or biol. effect. In another aspect of the present invention, a method is provided that induces a measure of tolerance to transgenic proteins, thus making longterm administration of the protein by gene therapy or recombinant protein possible and effective. In one embodiment of the invention, the ***mol*** .- ***switch*** , inducible-expression system comprises two nucleic acid or expression cassettes. The first expression cassette includes a ***promoter*** driving the expression of a ***mol*** . ***switch*** protein. The ***mol*** . ***switch*** protein is a chimeric or fusion protein that includes a mutated ***DNA*** ***binding*** domain characterized by a modification that eliminates a domain having a potential for autodimerization in the absence of an inducer while retaining those domains required for recognition of its cognate DNA sequence on the ***promoter*** of the second expression cassette. In one embodiment the ***DNA*** ***binding*** domain is a truncated GAL-4 ***DNA*** ***binding*** domain. The fusion protein further comprises a transactivation domain, and a mutated ligand-binding domain of a steroid-hormone receptor capable of being activated by a non-natural ligand inducer such as mifepristone. In a one embodiment, the ***promoter*** is a tissue-specific ***promoter*** such as .alpha.-actin ***promoter*** specific for muscle tissues. The first expression cassette may also include 5' untranslated regions, synthetic introns, and poly (A) signals that increase the fidelity and level of expression of the ***mol*** . ***switch*** gene. The second expression cassette includes a ***transgene*** encoding a desired gene product controlled by an inducible ***promoter*** comprising GAL-4 ***DNA*** - ***binding*** sites linked to a minimal ***promoter*** . The second expression cassette may also include 5' untranslated regions, synthetic introns, and poly (A) signals that increase the fidelity and level of expression of the ***transgene*** to be induced. In another embodiment of the invention, the inducible expression system is applied in vivo to effect expression of a ***transgene*** for gene therapy purposes. In one embodiment the inducible expression system is formulated with nonionic or anionic polymers and injected into an animal or human. Enhancement of transfection in vivo may be obtained with in vivo electroporation. The authors investigated the ability of an improved mifepristone-dependent GeneSwitch system to regulate the expression of genes for three therapeutic proteins: factor IX, IFN-.alpha., and erythropoietin. The GeneSwitch system consisted of two plasmids, one encoding the chimeric GeneSwitch protein, the other an inducible ***transgene*** . When the constitutive CMV ***promoter*** of the GeneSwitch plasmid was replaced by an autoinducible ***promoter*** consisting of four copies of ***GAL4*** ***DNA*** ***binding*** sites linked to a minimal thymidine kinase ***promoter*** , the tightness of ***transgene***

regulation was improved by an order of magnitude. Quant. RT-PCR anal. of GeneSwitch mRNA confirmed that the autoinducible ***promoter*** was responsive to mifepristone. The authors demonstrated the ability of the improved GeneSwitch system to regulate the expression of VEGF or erythropoietin in a biol. relevant manner after delivery of plasmids to the hindlimb muscle of adult mice. This ability of the autoinducible GeneSwitch system to regulate the expression of therapeutic proteins in mice indicates its potential for use in human gene therapy applications.

=> d his

(FILE 'HOME' ENTERED AT 12:54:51 ON 10 MAR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 12:55:38 ON 10 MAR 2003

```
L1          3988 S MOLECULAR SWITCH
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L3        391144 S (TRANSCRIPTION? FACTOR) OR (TRANSCRIPTION? REGULATORY PROTEIN
L4         46070 S VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 ORO
L5         74633 S VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 OR
L6         62751 S KRUPPEL OR KRAB OR KOX-1 OR TETR OR EVEN-SKIPPED OR LACR OR E
L7          1568 S RING1 OR SSB16 OR SSB24 OR TUP1 OR NAB1 OR AREB OR E4BP4 OR H
L8        137527 S L5 OR L6 OR L7
L9         71820 S TRANSGENE
L10           26 S (L1 OR L2) (P) L8 (P) L9
L11       243160 S (DNA BINDING)
L12       543375 S PROMOTER
L13          775 S (L1 OR L2) (P) L11 (P) L12
L14         5552 S NON-NATIVE
L15            0 S L13 (P) L14
L16            0 S L10 (P) L14
L17           10 DUPLICATE REMOVE L10 (16 DUPLICATES REMOVED)
L18            4 S L17 (P) L12 (P) L11
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=> s l17 not l18
L19 6 L17 NOT L18

=> d l19 1-6 ibib abs

L19 ANSWER 1 OF 6 MEDLINE

ACCESSION NUMBER: 2001667896 MEDLINE
DOCUMENT NUMBER: 21570561 PubMed ID: 11713335
TITLE: Modulation of myosin A expression by a newly established tetracycline repressor-based inducible system in Toxoplasma gondii.
AUTHOR: Meissner M; Brecht S; Bujard H; Soldati D
CORPORATE SOURCE: Zentrum fur Molekulare Biologie der Universitat Heidelberg, Im Neuenheimer Feld 282, 69102 Heidelberg, Germany.
SOURCE: NUCLEIC ACIDS RESEARCH, (2001 Nov 15) 29 (22) E115. Journal code: 0411011. ISSN: 1362-4962.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 20011120
Last Updated on STN: 20020125
Entered Medline: 20020111

AB We have developed a control system for regulating gene activation in Toxoplasma gondii. The elements of this system are derived from the Escherichia coli tetracycline resistance operon, which has been widely used to tightly control ***gene*** ***expression*** in eukaryotes. The tetracycline repressor ( ***tetR*** ) interferes with transcription initiation while the chimeric transactivator, composed of the ***tetR*** fused to the activating domain of ***VP16*** transcriptional factor, allows tet-dependent transcription. Accordingly, tetracycline derivatives such as anhydrotetracycline, which we found to be well tolerated by T.gondii, can serve as effector molecules, allowing control of ***gene*** ***expression*** in a reversible manner. As a prerequisite to functionally express the ***tetR*** in T.gondii, we used a synthetic gene with change of codon frequency. Whereas no

activation of transcription w[redacted] achieved using the synthetic tetracycline-controlled trans[redacted]ivator, tTA2(s), the ***TetR[redacted] (s ) ***modulates*** parasite transcription over a range of approximately 15-fold as measured for several reporter genes. We show here that the ***tetR*** -dependent induction of the T.gondii myosin A ***transgene*** expression drastically down-regulates the level of endogenous MyoA. This myosin is under the control of a tight feedback mechanism, which occurs at the protein level.

L19 ANSWER 2 OF 6 MEDLINE
ACCESSION NUMBER: 2001552483 MEDLINE
DOCUMENT NUMBER: 21475980 PubMed ID: 11591893
TITLE: Design and in vitro characterization of a single regulatory module for efficient control of gene expression in both plasmid DNA and a self-inactivating lentiviral vector.
AUTHOR: Ogueta S B; Yao F; Marasco W A
CORPORATE SOURCE: Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA.
CONTRACT NUMBER: AI28785 (NIAID)
AI41954 (NIAID)
CA06516 (NCI)
SOURCE: MOLECULAR MEDICINE, (2001 Aug) 7 (8) 569-79.
Journal code: 9501023. ISSN: 1076-1551.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20011016
Last Updated on STN: 20020215
Entered Medline: 20020214

AB BACKGROUND: Regulation of ***transgene*** expression in target cells represents a critical and challenging aspect of gene therapy. Recently, a two-plasmid tetracycline-inducible system was developed in which the tetracycline repressor ( ***tetR*** ) alone, rather than the ***tetR*** - ***VP16*** fusion derivative, was shown to function as a potent trans- ***modulator*** of a second plasmid that contains two tandem repeats of the tetracycline operator (tetO) inserted between the TATA box and the transcription start site of the hCMV major immediate-early promoter. A technological advance in this area would be the development of a single autoregulatory cassette that incorporates both of these components into nonviral and viral gene transfer vectors. For the latter, an inducible lentiviral vector that is capable of temporal and quantitative control of ***gene*** ***expression*** in either dividing or nondividing cells is highly desirable. MATERIALS AND METHODS: A one-piece inducible (1Pi) autoregulatory cassette was constructed to provide IRES-mediated translation of the ***tetR*** as well as tight control over the tetO unit preventing transcription initiation of the first cistron in the absence of the tetracycline. To increase efficiency of ***tetR*** -mediated repression, a nuclear localization signal was incorporated at the 3' end of the ***tetR*** gene. Regulation of ***gene*** ***expression*** at the transcriptional and protein level was analyzed in transient transfection experiments using plasmid DNA. Construction of a self-inactivating lentiviral vector containing this 1Pi cassette allowed the study of its long-term effectiveness in primary human cells. RESULTS: The 1Pi autoregulatory cassette when incorporated into plasmid DNA allows efficient control of the secretable hEGF as well as eGFP expression in a variety of cell types. Transient transfection studies demonstrated that the time course of repression is different for the 1Pi and two-plasmid system (2Pi). In the 2Pi system, greater repression is seen with the first 24-48 hr; however, by 72 hr, similar levels of repression with the 1Pi and 2Pi systems are obtained. This regulation is reached three times faster when the ***tetR*** is modified with a nuclear localization signal to direct nascent proteins into the nuclear compartment. In addition, stable transduction of human umbilical vein endothelial cells (HUVEC) with a self-inactivating lentiviral vector incorporating this single regulator cassette provided tetracycline-inducible control of ***gene*** ***expression*** that is not diminished over time and is completely reversible upon removal of tetracycline. CONCLUSIONS: These results suggest a model in which the 1Pi autoregulatory system reaches a steady state over time, the minimal amount

of ***tetR*** produced by the basal activity of the CMV promoter and accumulated is adequate to re[redacted]ce the ***tetR*** that is [redacted]t over time. These studies also show that the inducible self-inactivating lentiviral vector can temporally and reversibly regulate ***transgene*** expression in HUVECs. The use of this transcriptional control unit in both nonviral and viral vector delivery systems will constitute an attractive technological advance for many gene therapy applications where temporal and quantitative control of ***gene*** ***expression*** is desired. The strengths and limitations of the 1Pi system are discussed.

L19 ANSWER 3 OF 6 MEDLINE
ACCESSION NUMBER: 1999013445 MEDLINE
DOCUMENT NUMBER: 99013445 PubMed ID: 9799101
TITLE: Protein kinase Cmu downregulation of tumor-necrosis-factor-induced apoptosis correlates with enhanced expression of nuclear-factor-kappaB-dependent protective genes.
AUTHOR: Johannes F J; Horn J; Link G; Haas E; Siemienski K; Wajant H; Pfizenmaier K
CORPORATE SOURCE: Institute of Cell Biology and Immunology, University of Stuttgart, Germany.. Franz-Josef.Johannes@po.uni-Stuttgart.de
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1998 Oct 1) 257 (1) 47-54.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981110

AB Protein kinase Cmu (PKCmu) represents a new subtype of the PKC family characterized by the presence of a pleckstrin homology (PH) domain and an amino-terminal hydrophobic region. In order to analyse the potential role of PKCmu in signal-transduction pathways, stable PKCmu transfectants were established with human and murine cell lines. All transfectants showed a reduced sensitivity to tumor-necrosis-factor (TNF)-induced apoptosis, which correlated with the amount of ***transgene*** expressed and with an enhanced basal transcription rate of ***NF*** - ***kappaB*** -driven genes including the inhibitor of apoptosis protein 2 (cIAP2) and TNF-receptor-associated protein 1 (TRAF1). Sensitivity to apoptosis induced by the lipid mediator ceramide was unchanged in PKCmu transfectants. In support of a PKCmu action on ***NF*** - ***kappaB*** , we show enhancement and downregulation of TNF-induced expression of a ***NF*** - ***kappaB*** -dependent reporter gene by transient overexpression of wild-type and kinase-negative mutants of PKCmu, respectively. Interestingly, no significant changes were found in an electrophoretic mobility shift assay, indicative of PKCmu action downstream of IkappaB degradation, probably by ***modulation*** of the transactivation capacity of ***NF*** - ***kappaB*** . The dominant negative action of the kinase-negative mutant further suggest a regulatory role of PKCmu for ***NF*** - ***kappaB*** -dependent ***gene*** ***expression*** .

L19 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:576423 CAPLUS
DOCUMENT NUMBER: 129:286539
TITLE: Tetracycline repressor, tetR, rather than the tetR-mammalian cell transcription factor fusion derivatives, regulates inducible gene expression in mammalian cells
AUTHOR(S): Yao, Feng; Svensjo, Tor; Winkler, Thomas; Lu, Michael; Eriksson, Carl; Eriksson, Elof
CORPORATE SOURCE: Laboratory of Tissue Repair and Gene Transfer, Division of Plastic Surgery, Brigham and Women's Hospital, Boston, MA, 02115, USA
SOURCE: Human Gene Therapy (1998), 9(13), 1939-1950
CODEN: HGTHE3; ISSN: 1043-0342
PUBLISHER: Mary Ann Liebert, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB This article describes a tetracycline-inducible regulatory system that demonstrates that the tetracycline repressor ( ***tetR*** ) alone, rather than ***tetR*** -mammalian cell transcription factor fusion derivs., can function as a potent trans- ***modulator*** to regulate ***gene*** ***expression*** in mammalian cells. With proper positioning of tetracycline operators downstream of the TATA element and of human epidermal growth factor (hEGF) as a reporter, we show that ***gene*** ***expression*** from the tetracycline operator-bearing hCMV major immediate-early enhancer-promoter (pcmvtetO) can be regulated by ***tetR*** over three orders of magnitude in response to tetracycline when (1) the reporter was cotransfected with ***tetR*** -expressing plasmid in transient expression assays, and (2) the reporter unit was stably integrated into the chromosome of a ***tetR*** -expressing cell line. This level of ***tetR*** -mediated inducible gene regulation is significantly higher than that of other repression-based mammalian cell transcription switch systems. In an in vivo porcine wound model, close to 60-fold ***tetR*** -mediated regulatory effects were detected and it was reversed when tetracycline was administered. Collectively, this study provides a direct implementation of this tetracycline-inducible regulatory switch for controlling ***gene*** ***expression*** in vitro, in vivo, and in gene therapy. Regulation of ***transgene*** expression in target cells represents a crit. and challenging aspect of gene therapy. Using the hCMV major immediate-early promoter as a prototype mammalian cell promoter, this study demonstrates that ***tetR*** alone, rather than the previously used ***tetR*** -mammalian cell transcription factor fusion derivs., can function as a potent repressor of expression of genes under the tet operator-contg. hCMV major immediate-early promoter, while its natural promoter activity is preserved. Specifically, with hEGF as a secretable reporter, more than 1000-fold tetracycline-reversible regulation can be detected in transient transfection assays, and in ***tetR*** -expressing stable cell lines with a chromosomally integrated hEGF reporter unit. These observations suggest a direct implementation of this biol. switch in regulating the expression of ***transgenes*** in cell biol., mol. virol., and gene therapy.
REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 5 OF 6 SCISEARCH COPYRIGHT 2003 ISI (R)
ACCESSION NUMBER: 2001:949910 SCISEARCH
THE GENUINE ARTICLE: 496CK
TITLE: Modulation of myosin A expression by a newly established tetracycline repressor-based inducible system in Toxoplasma gondii
AUTHOR: Meissner M; Brecht S; Bujard H; Soldati D (Reprint)
CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biol Sci, Imperial Coll Rd, London SW7 2AZ, England (Reprint); Univ Heidelberg, Zentrum Mol Biol, D-69102 Heidelberg, Germany
COUNTRY OF AUTHOR: England; Germany
SOURCE: NUCLEIC ACIDS RESEARCH, (15 NOV 2001) Vol. 29, No. 22, pp. U58-U67.
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0305-1048.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 26
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB We have developed a control system for regulating gene activation in Toxoplasma gondii. The elements of this system are derived from the Escherichia coli tetracycline resistance operon, which has been widely used to tightly control ***gene*** ***expression*** in eukaryotes. The tetracycline repressor ( ***tetR*** ) Interferes with transcription initiation while the chimeric transactivator, composed of the ***tetR*** fused ito the activating domain of ***VP16*** transcriptional factor, allows tet-dependent transcription. Accordingly, tetracycline derivatives such as anhydrotetracycline, which we found to be well tolerated by T.gondii, can serve as effector molecules, allowing control of ***gene*** ***expression*** in a reversible manner. As a prerequisite to functionally express the ***tetR*** in T.gondii, we used a synthetic gene with change of codon frequency. Whereas no

activation of transcription w[redacted] achieved using the synthetic tetracycline-controlled trans[redacted]ivator, tTA2(s), the ***Tet[redacted]* (s) ***modulates*** parasite transcription over a range of similar to 15-fold as measured for several reporter genes. We show here that the ***tetR*** -dependent induction of the T.gondii myosin A ***transgene*** expression drastically down-regulates the level of endogenous MyoA. This myosin is under the control of a tight feedback mechanism, which occurs at the protein level.

L19 ANSWER 6 OF 6 SCISEARCH COPYRIGHT 2003 ISI (R)
ACCESSION NUMBER: 2001:455320 SCISEARCH
THE GENUINE ARTICLE: 436EW
TITLE: A regulated, NF kappa B-assisted import of plasmid DNA into mammalian cell nuclei
AUTHOR: Mesika A; Grigoreva I; Zohar M; Reich Z (Reprint)
CORPORATE SOURCE: Weizmann Inst Sci, Dept Biol Chem, IL-76100 Rehovot, Israel (Reprint); Biotechnol Gen, Div Res, IL-76100 Rehovot, Israel
COUNTRY OF AUTHOR: Israel
SOURCE: MOLECULAR THERAPY, (MAY 2001) Vol. 3, No. 5, Part 1, pp. 653-657.
Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA.
ISSN: 1525-0016.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 32

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The success of synthetic DNA delivery systems in human gene therapy will be enhanced by increasing transfection efficiencies and by providing tighter control over targeting of the DNA into the nucleus. Here, we used DNA vectors that contain repetitive binding sites for the inducible transcription factor ***NF*** ***kappaB*** , which is transported into the nucleus by the nuclear import machinery. Nuclear entry of the modified vectors was augmented 12-fold and was associated with corresponding increase in ***gene*** ***expression*** . Depending on their position, the binding sites could also function as transcriptional enhancers, increasing ***gene*** ***expression*** levels up to an additional 19-fold. Notably, nuclear targeting of the DNA and ***transgene*** transcription could both be regulated by exogenous stimulators which ***modulate*** the intracellular distribution of ***NF*** ***kappaB*** . The approach provides a framework for the controlled targeting of constitutive or transcriptionally regulated synthetic vectors into mammalian cell nuclei.

=> d his

(FILE 'HOME' ENTERED AT 12:54:51 ON 10 MAR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 12:55:38 ON 10 MAR 2003

| | | | |
|---|---|---|---|
| L1 | 3988 | S | MOLECULAR SWITCH |
| L2 | 35784 | S | MODULAT? (P) (GENE EXPRESSION) |
| L3 | 391144 | S | (TRANSCRIPTION? FACTOR) OR (TRANSCRIPTION? REGULATORY PROTEIN |
| L4 | 46070 | S | VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 ORO |
| L5 | 74633 | S | VP16 OR NF-KAPPAB OR GAL4 OR TFE3 OR ITF1 OR OCT-1 OR SP1 OR |
| L6 | 62751 | S | KRUPPEL OR KRAB OR KOX-1 OR TETR OR EVEN-SKIPPED OR LACR OR E |
| L7 | 1568 | S | RING1 OR SSB16 OR SSB24 OR TUP1 OR NAB1 OR AREB OR E4BP4 OR H |
| L8 | 137527 | S | L5 OR L6 OR L7 |
| L9 | 71820 | S | TRANSGENE |
| L10 | 26 | S | (L1 OR L2) (P) L8 (P) L9 |
| L11 | 243160 | S | (DNA BINDING) |
| L12 | 543375 | S | PROMOTER |
| L13 | 775 | S | (L1 OR L2) (P) L11 (P) L12 |
| L14 | 5552 | S | NON-NATIVE |
| L15 | 0 | S | L13 (P) L14 |
| L16 | 0 | S | L10 (P) L14 |
| L17 | 10 | | DUPLICATE REMOVE L10 (16 DUPLICATES REMOVED) |
| L18 | 4 | S | L17 (P) L12 (P) L11 |
| L19 | 6 | S | L17 NOT L18 |

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